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Improving anoxic/aerobic nutrients removal by the enhanced biological phosphorus removal-sulfur autotrophic denitrification (EBPR-SAD) system when treating low C/N ratio municipal wastewater

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ABSTRACT

A novel nutrients removal system integrating enhanced biological phosphorus removal (EBPR) and sulfur autotrophic denitrification (SAD) was developed to upgrade the Shenyang Degremont Anoxic Oxic process (SDAO). In this system, the EBPR process was mainly employed to utilize organic carbon for denitrification and phosphorus removal; the SAD process was used to remove nitrate, which was not removed in the EBPR process because of a low C/N ratio. The results showed that the EBPR-SAD effluent COD, TN, NH_4^+–N and TP were 24.6, 1.21, 1.09 and 0.24 mg/L, respectively. Compared with those of the original system, the removal efficiencies of TN and TP increased to 95.8% and 86.9%. It was demonstrated that the EBPR-SAD system could achieve nearly complete nutrients removal from low C/N ratio municipal wastewater. Cluster analysis and principal coordinate analysis showed that bacterial community structures were significantly different between SDAO, EBPR and SAD processes, indicating that bacterial community structures were affected by the type of wastewater biotreatment system. Taxonomic analysis showed that the nine most abundant phyla in the SDAO and EBPR-SAD system accounted for 87.0%–90.7% of the total effective sequences. Redundancy analysis was used to reveal the relationship between the abundance of bacterial phyla and environmental parameters in the SDAO and EBPR-SAD system.

Keywords: EBPR-SAD system; Low C/N ratio wastewater; Efficient nutrients removal; Material balance calculation; High-throughput sequencing; Redundancy analysis

1. Introduction

Excessive discharge of pollutant nutrients can cause eutrophication of water environments and deterioration of water quality and is a potential hazard to human health [1–3]. Biological nutrient removal (BNR) processes are generally accepted and widely applied in existing wastewater treatment plants (WWTPs) because of their economic superiority [4,5]. However, BNR processes are usually unreliable in simultaneously removing nitrogen and phosphorus because

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there are three main constraints in anaerobic/anoxic/aerobic systems: a shortage of organic carbon in the WWTP influent; NO_3^-–N in the return sludge and the sludge retention time (SRT) between nitrifiers and polyphosphate accumulating organisms (PAOs) [6–8]. Efficient nutrients removal is difficult for conventional anaerobic/anoxic/aerobic processes in treating low C/N ratio municipal wastewater.

When the C/N ratio of municipal wastewater is approximately 6–7, conventional nitrification–denitrification processes can obtain excellent nutrients removal performance using step-feed technology, such as the Johannesburg (JHB) or anoxic/oxic sequencing batch reactors process [5,9]. However, nutrients removal performance will deteriorate if the C/N ratio is less than 6–7. When the C/N ratio is greater than 4, shortcut nitrification–denitrification processes, such as the signal reactor for high activity ammonia removal over nitrite process, are able to achieve efficient nutrient removal [10,11]. However, the application of shortcut nitrification–denitrification processes is restricted by the need for precise operational conditions, including dissolved oxygen (DO), temperature, pH and alkalinity [12–15]. On the other hand, nitrogen removal efficiency cannot reach 100% using conventional nitrification–denitrification or shortcut nitrification–denitrification processes under anoxic/aerobic conditions, even if the influent organic carbon is sufficient for the removal of nitrogenous pollutants. Nitrogen removal efficiency, which is calculated by $(r + R)/(r + R + 1)$, depends on the nitrate recycling ratio, r, and the sludge return ratio, R. Thus, developing efficient nutrient removal technology for treating low C/N ratio municipal wastewater has attracted increasing attention and become particularly important [16–19].

Recently, an efficient nitrogen removal system integrating the JHB process and the sulfur autotrophic denitrification (SAD) process was proposed to treat low C/N ratio municipal wastewater [20]. In this wastewater treatment system, the JHB process was mainly employed for nitrogen removal via nitrification–heterotrophic denitrification, and afterwards, the SAD process was proposed to further remove nitrate that was not removed in the JHB process because of organic carbon shortage. Under the SAD process, sulfur served as an electron donor, and nitrate was reduced by bacteria via autotrophic denitrification. The average nitrogen removal efficiency of the JHB-SAD system reached 95% under an influent C/N ratio of 4–5. However, phosphorus removal was not examined. Moreover, the nitrogen removal capacity of the anoxic zone in the JHB-SAD system was inefficient because of the organic carbon shortage, which resulted in wasted space. Therefore, a more efficient nutrient removal system integrating enhanced biological phosphorus removal (EBPR) and SAD processes was proposed in this study. Compared with the JHB-SAD system, the anoxic zone and nitrate recycling were abandoned in the EBPR-SAD system. In this novel system, the EBPR process was mainly used for phosphorus removal, nitrification and heterotrophic denitrification, and the SAD process was used for removal of nitrates that were not removed by heterotrophic denitrification. Therefore, the EBPR-SAD system offers the potential advantage of solving the electron donor shortage problem and achieving efficient nutrients removal when treating low C/N ratio municipal wastewater.

In this study, the EBPR-SAD system was proposed to improve an anoxic/aerobic nutrients removal system (Shenyang Degremont Anoxic Oxic process ([SDAO). The organic carbon and nutrients pollutant removal performances of these two systems were compared when treating low C/N ratio municipal wastewater. Based on materials balance, nutrients removal mechanisms were also examined. At the same time, high-throughput sequencing, which is considered to be a powerful method for investigating the microbial community in sludge samples of various wastewater treatment processes [21–24], was carried out on the SDAO and EBPR-SAD systems to assess the bacterial communities. Cluster analysis (CA) and principal coordinate analysis (PCoA) were used to assess the community diversity among the SDAO and EPBR-SAD systems, and redundancy analysis (RDA) was used to determine correlations between the abundance of phyla and environmental parameters. The objective of this study was to provide a sound understanding of the novel EBPR-SAD system for the treatment of municipal wastewater with a low C/N ratio.

2. Experimental methods and materials

2.1. Experimental system setup

A pilot-scale SDAO system was established based on the existing full-scale SDAO system at the Shenyang North Wastewater Treatment Plant. The SDAO system consisted of a sludge restore zone (SR, working volume of 0.83 m^3), an anoxic zone (ANO, working volume of 1.16 m^3), an aerobic zone (AE, working volume of 2.77 m^3) and a secondary settler (SES, working volume of 1.07 m^3), as shown in Figs. 1(a) and (b). The SDAO system can be described as follows: first, return sludge flows into the sludge recovery zone, which is recovered by microaeration; then, return sludge flows into the anoxic zone, which is mixed and reacted with nitrate recycling and influent; finally, the mixture flows into the aerobic zone, and nitrification is realized by aeration.

According to the Discharge Standards of Pollutants for Municipal Wastewater Treatment Plants in China (GB-18918-2002), the Shenyang North Wastewater Treatment Plant needs an upgrade to achieve effective nutrients pollutant removal. Thus, the EBPR-SAD system was explored as an upgrade to the SDAO system. The layout of the EBPR-SAD system is shown in Figs. 1(c) and (d). To reduce costs, the infrastructure for EBPR was transformed from existing SDAO structures. The sludge recovery zone and the anoxic zone were converted to a pre-anoxic zone (PAN) and an anaerobic zone (ANA). The secondary settler effluent from the EBPR process was pumped into the SAD reactor. The height and inner diameter of the up-flow SAD reactor were 75 cm and 14 cm, respectively. The packing height of the SAD reactors was 70 cm. The SAD reactor was filled with granular sulfur and limestone with equivalent diameters of 3–4 mm and 0.8–1.2 mm (mass ratio 2:1), respectively; the porosity of the SAD reactor was 45.7%.

To compare the performances of the two wastewater treatment systems, both the SDAO and EBPR-SAD systems were operated under the same conditions. The inflow was 0.56 m^3/h during the different experiment periods. The nitrate recycling ratio (r) and sludge return ratio (R) were set to 200% and 100%, respectively, in the SDAO process. The hydraulic retention times (HRTs) of the different zones were 1.49 h (sludge recovery zone), 2.08 h (anoxic zone), 4.95 h (aerobic zone) and 1.91 h (secondary settler) in the SDAO system.

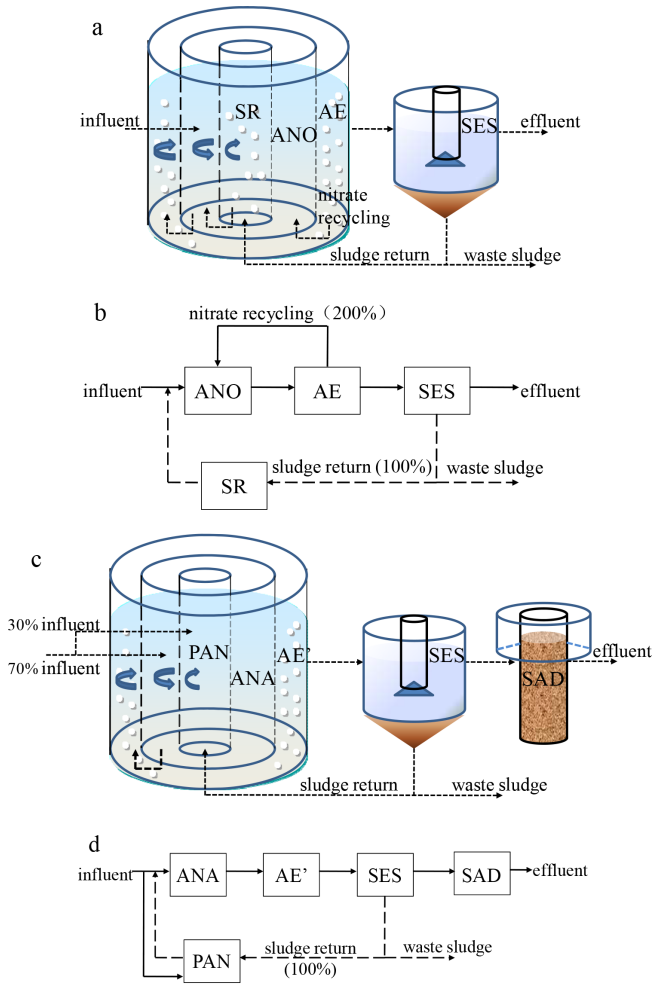

Fig. 1. Layout and schematic diagram of the SDAO system (a and b) and EBPR-SAD system (c and d). (SR: sludge restore zone; ANO: anoxic zone; AE: aerobic zone in SDAO system; SES: secondary settler; PAN: pre-anoxic zone; ANA: anaerobic zone; AE': aerobic zone in EBPR-SAD system; SAD: sulfur autotrophic denitrification reactor.)

In the EBPR-SAD system, the influent distribution ratios of the pre-anoxic and ANAs were 30% and 70%, respectively. Moreover, nitrate recycling was eliminated to ensure a strict anaerobic environment in the ANA. The HRTs were 1.49 h (PAN), 2.88 h (ANA), 4.95 h (aerobic zone) and 1.91 h (secondary settler) in the EBPR process. The actual hydraulic retention time (AHRT) of the SAD reactor was 10 min.

Mixed liquor suspended solids (MLSS) were controlled to fall within 2,500–2,700 mg/L. The SRT was controlled to within 17–23 d, with sodium acetate dosing and 27–33 d for normal operation. The DO concentration in the sludge recovery zone (phase I–II only) was controlled to within 0.4–0.8 mg/L, and that in the aerobic zone was controlled to within 2.0–2.5 mg/L (phase I–IV).

2.2. Wastewater source and sludge

The experiment was conducted at the Guodian Shenyang Northern Wastewater Treatment Plant in Shenyang, Liaoning Province, China. Experimental raw wastewater was pumped from the WWTP sewer line. The seed sludge was collected from the full-scale SDAO wastewater treatment system where nutrients removal was inefficient. The influent C/N ratio was approximately 4–5, which presents typically low C/N ratio municipal wastewater.

2.3. Analytical methods

Nitrate (NO_3^-–N), ammonia (NH_4^+–N), total nitrogen (TN), total phosphate (TP) and MLSS were analyzed according to standard methods [25]. The chemical oxygen demand (COD) was measured by a Lianhua COD quick-analysis apparatus (Lianhua Technology Co., Ltd., China). The DO was determined by WTW Oxi 3310 meters with DO probes.

2.4. Experimental procedure

The pilot-scale wastewater treatment facility was operated for a period of 127 d, which included four experimental phases. Phase I (days 1–31) was used to compare the performance of the pilot-scale SDAO system with that of the full-scale SDAO system. Phase II (days 32–60) was used to investigate the nutrients removal performance of the pilot-scale SDAO system with sufficient organic carbon (influent C/N ratio increased to 7.8 by adding sodium acetate). Sewage flowed into the anoxic zone, and the nitrate recycling ratio (r) and sludge return ratio (R) were set to 200% and 100% when the experimental system performed the SDAO process. Phase III (days 61–93) was used to evaluate the performance and feasibility of the EBPR-SAD system for nutrients removal from low C/N ratio municipal wastewater. Phase IV (days 94–127) was used to improve phosphorus removal by adding sodium acetate (COD of 40 mg/L) into the EBPR-SAD system influent. Approximately, 30% influent flowed into the PAN and 70% influent flowed into the ANA when the experimental system was conducting the EBPR-SAD process. At the same time, nitrate recycling was eliminated, and the sludge return ratio (R) was set to 100%.

2.5. Calculations of pollutant removal capacity in different zones

Based on materials balance, the COD, nitrogen and TP removal capacity along the SDAO system were calculated using the following equations:

$$\text{Sludge recovery zone: } Q_R C_{SES.C,N,P} = Q_R C_{SR.C,N,P} + \Delta S_{SR.C,N,P} \quad (1)$$

$$\text{Anoxic zone: } Q_R C_{SR.C,N,P} + Q_{inf} C_{inf.C,N,P} + Q_r C_{AE.C,N,P} = (Q_R + Q_{inf} + Q_r) C_{ANO.C,N,P} + \Delta S_{ANO.C,N,P} \quad (2)$$

$$\text{Aerobic zone: } (Q_R + Q_{inf} + Q_r) C_{ANO.C,N,P} = (Q_R + Q_{inf} + Q_r) C_{AE.C,N,P} + \Delta S_{AE.C,N,P} \quad (3)$$

$$\text{Secondary settler: } (Q_R + Q_{inf}) C_{AE.C,N,P} = (Q_R + Q_{inf}) C_{SES.C,N,P} + \Delta S_{SES.C,N,P} \quad (4)$$

The COD, nitrogen and TP removal capacity along the EBPR-SAD system were calculated using the following equations:

$$\text{Pre-anoxic zone: } 30\%\ Q_{inf}\ C_{inf.C,N,P} + Q_R\ C_{SES.C,N,P} = (30\%\ Q_{inf} + Q_R)\ C_{PAN.C,N,P} + \Delta S_{PAN.C,N,P} \quad (5)$$

$$\text{Anaerobic zone: } 70\% Q_{inf}\ C_{inf.C,N,P} + (30\%\ Q_{inf} + Q_R)\ C_{PAN.C,N,P} = (Q_{inf} + Q_R)\ C_{ANA.C,N,P} + \Delta S_{ANA.C,N,P} \quad (6)$$

$$\text{Aerobic zone: } (Q_{inf} + Q_R)\ C_{ANA.C,N,P} = (Q_{inf} + Q_R)\ C_{AE.C,N,P} + \Delta S_{AE.C,N,P} \quad (7)$$

$$\text{Secondary settler: } (Q_{inf} + Q_R)\ C_{AE.C,N,P} = (Q_{inf} + Q_R)\ Q_{SES.C,N,P} + \Delta S_{SES.C,N,P} \quad (8)$$

$$\text{SAD reactor: } Q_{inf}\ C_{SES.C,N,P} = Q_{inf}\ C_{SAD.C,N,P} + \Delta S_{SAD.C,N,P} \quad (9)$$

where Q_{inf}, Q_R and Q_r are the influent flows, sludge return flow and nitrate recycling flow, respectively; $C_{inf.C,N,P}$, $C_{SR.C,N,P}$, $C_{ANO.C,N,P}$, $C_{AE.C,N,P}$, $C_{SES.C,N,P}$ $C_{PAN.C,N,P}$, $C_{ANA.C,N,P}$, and $C_{SAD.C,N,P}$ represent the pollutants' concentrations in the influent, sludge recovery zone, anoxic zone, aerobic zone, secondary settler, PAN, ANA and SAD reactor, respectively; ΔS_{SR}, $\Delta S_{ANO.C,N,P}$, $\Delta S_{AE.C,N,P}$, $\Delta S_{SES.C,N,P}$, $\Delta S_{PAN.C,N,P}$, $\Delta S_{ANA.C,N,P}$, and $\Delta S_{SAD.C,N,P}$ denote the pollutant changed capacity in the sludge recovery zone, anoxic zone, aerobic zone, secondary settler, PAN, ANA and SAD reactor, respectively; and 30% and 70% are the influent flow ratios. As shown in Fig. 4, Eqs. (1)–(9) were used to calculate the capacity of COD, nitrogen and TP change in different units of the SDAO and EBPR-SAD systems.

2.6. Bacterial community analysis

2.6.1. DNA extraction, PCR amplification and sequencing

To investigate the bacterial communities in the SDAO and EBPR-SAD systems, sludge samples from the two systems were collected on days 58 and 125, respectively. For sludge samples, genomic DNA was extracted using an E.Z.N.A. Soil DNA Kit D5625-01 (Omega, USA) according to the manufacturer's protocol. The extracted DNA concentration was quantified by a Qubit2.0 DNA Assay Kit Q10212 (Life, USA). The integrity of the extracted DNA was detected by 1.5% agarose gel electrophoresis.

The V3-V4 region of 16S rRNA genes was amplified from the genomic DNA via primer pairs 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) for bacteria. The nucleotide barcodes that allow for sample multiplexing during sequencing were inserted between the Miseq adaptors and the forward primers. The first round of PCR reactions was carried out in a 50 μL PCR mixture comprising 5 μL of 10X PCR buffer, 0.5 μL of 10 nM dNTPs, 10 ng of genomic DNA, 0.5 μL of each primer (50 μM), 0.5 μL of 5 U/μL Platinum Taq DNA Polymerase Ep0406 (Thermo Fisher Scientific, USA), and ddH_2O added to 50 μL. The conditions of the first-round PCR were as follows: 94°C for 3 min; followed by 5 cycles at 94°C for 30 s, 45°C for 20 s and 65°C for 30 s; then 20 cycles at 94°C for 20 s, 55°C for 20 s and 72°C for 30 s; and a final extension at 72°C for 5 min. The second-round PCR reactions were then carried out in a 50 μL PCR mixture containing 5 μL of 10X PCR buffer, 0.5 μL of 10 nM dNTPs, 20 ng of DNA, 0.5 μL of each primer (50 μM), 0.5 μL of 5 U/μL Platinum Taq DNA Polymerase Ep0406 (Thermo Fisher Scientific, USA) and ddH_2O added to 50 μL. The conditions for the second-round PCR were as follows: 95°C for 30 s; followed by 5 cycles at 95°C for 15 s, 55°C for 15 s, and 72°C for 30 s; and a final extension at 72°C for 5 min.

The PCR products were pooled and purified by a SanPrep DNA Gel Extraction Kit SK8192 (Sangon Biotech, China), and the concentrations of the purified products were quantified by a Qubit2.0 DNA Assay Kit Q10212 (Life, USA). The purified products were then mixed in equal amounts based on DNA concentration, followed by sequencing on the MiSeq sequencing platform (Illumina, USA) at Sangon Biotech Co., Ltd., (Shanghai, China). After sequencing, the low-quality sequences, adapter primers, barcodes and primers were removed from the resulting sequences. The treated sequences were then examined, and the chimeric sequences were filtered out by Chimera Slayer in the Mothur package. The remaining sequences formed effective sequences. The sequencing produced 26,248 (sludge recovery zone), 24,827 (anoxic zone) and 22,770 (aerobic zone) high-quality reads for the SDAO system and 27,871 (PAN), 24,855 (ANA), 29,473 (aerobic zone) and 26,093 (SAD reactor) high-quality reads for the EBPR-SAD system.

2.6.2. Biodiversity analysis and phylogenetic taxonomy

The effective sequences were clustered into operational taxonomic units (OTUs) by setting the similarity to 97% using the Ribosomal Database Project (RDP) classifier. Based on the OTUs, coverage percentage, abundance-based coverage estimator (ACE), Chao1 richness estimator and Shannon diversity indices were calculated in the Mothur software package for each sample. Based on the UniFrac metric, Beta diversity statistics, including CA and PCoA, were carried out to assess the community diversity among the SDAO and EPBR-SAD systems. To identify the species, representative sequences from each OTU were compared with the entries in the SILVA database with a confidence threshold of 80%. In addition, RDA was used to assess the relationship between the abundance of phyla and the environmental parameters via R software [26].

3. Results

3.1. Performance of pollutant removal in the SDAO and EBPR-SAD systems

Because the SDAO system could not achieve excellent nutrients removal performance, the biotreatment system needed to be upgraded. Based on the characteristics of the influent and effluent in the SDAO system, the sludge recovery zone and anoxic zone in the SDAO system were converted to pre-anoxic and ANAs for an EBPR process. Approximately 30% influent flowed into the PAN to eliminate NO_3^-–N in the return sludge. Approximately 70% influent flowed into the ANA for PAOs to release phosphorus. Nitrate recycling was eliminated to ensure an anaerobic environment in the EBPR process. At the same time, the SAD process was proposed to treat NO_3^-–N from the EBPR process effluent and further improve nitrogen removal performance. The COD and nutrients pollutant removal performances of the SDAO and EBPR-SAD systems during phases I–IV are presented in Figs. 2 and 3.

3.1.1. COD removal

In phases I–IV, the influent COD values were 172.5, 237.3, 140.4 and 184.8 mg/L on average, as shown in Fig. 2(a). The effluent COD values in the SDAO and EBPR-SAD systems were 38.1, 34.6, 24.6 and 24.6 mg/L on average, and the corresponding removal efficiencies were 77.9%, 85.4%, 82.5% and 86.7% during phases I, II, III and IV, respectively. The variations in COD throughout the SDAO and EBPR-SAD systems are presented in Fig. 3. The COD concentration decreased rapidly when sewage flowed into those two wastewater treatment systems and was approximately 24.5–40.1 mg/L (Fig. 3). The results indicated that the COD was maintained at a low level and did not change significantly in either system, although the structure was transformed when upgrading the SDAO system to an EBPR-SAD system. In addition, the COD in the SAD reactor effluent was approximately 7 mg/L lower than in the secondary settler effluent (phase III–IV), which may be attributed to the filtration of sulfur granules.

3.1.2. Nitrogen removal

In phases I–IV, the nitrogen pollutant removal performance of the SDAO and EBPR-SAD systems was investigated, and the results are shown in Figs. 2(b) and (c) and 3. The systems' effluent NH_4^+–N levels were, on average, 1.49 mg/L (SDAO system) and 1.11 mg/L (EBPR-SAD system) with a corresponding removal efficiencies of 94.1% and 95.8%, when influent NH_4^+–N concentrations were approximately 19.4–34.6 mg/L (Fig. 2(b)). Although the wastewater treatment process was upgraded from an SDAO to an EBPR-SAD system, the NH_4^+–N removal performances of the two systems were analogous, and efficient NH_4^+–N removal was achieved during phases I–IV. In addition, approximately 0.5 mg/L NH_4^+–N was removed under the SAD process because of the assimilation of sulfur autotrophic bacteria, as shown in Figs. 2(b) and 3.

Compared with the NH_4^+–N removal performance, the TN removal performance of the SDAO process was disappointing during phase I, as shown in Figs. 2(c) and 3(a). Under an influent TN of 33.0 mg/L, the TN removal efficiency was only 26.2%, with 24.4 mg/L in the effluent. Because the influent C/N ratio was approximately 4–5, denitrification was weak in the SDAO system. NO_3^-–N was the predominant compound in the SDAO system effluent, with a significant increase to 21.4 mg/L (Fig. 3(a)). To improve TN removal, sodium acetate served as an external organic carbon source and was added into the influent, increasing the C/N ratio to 7.8 during phase

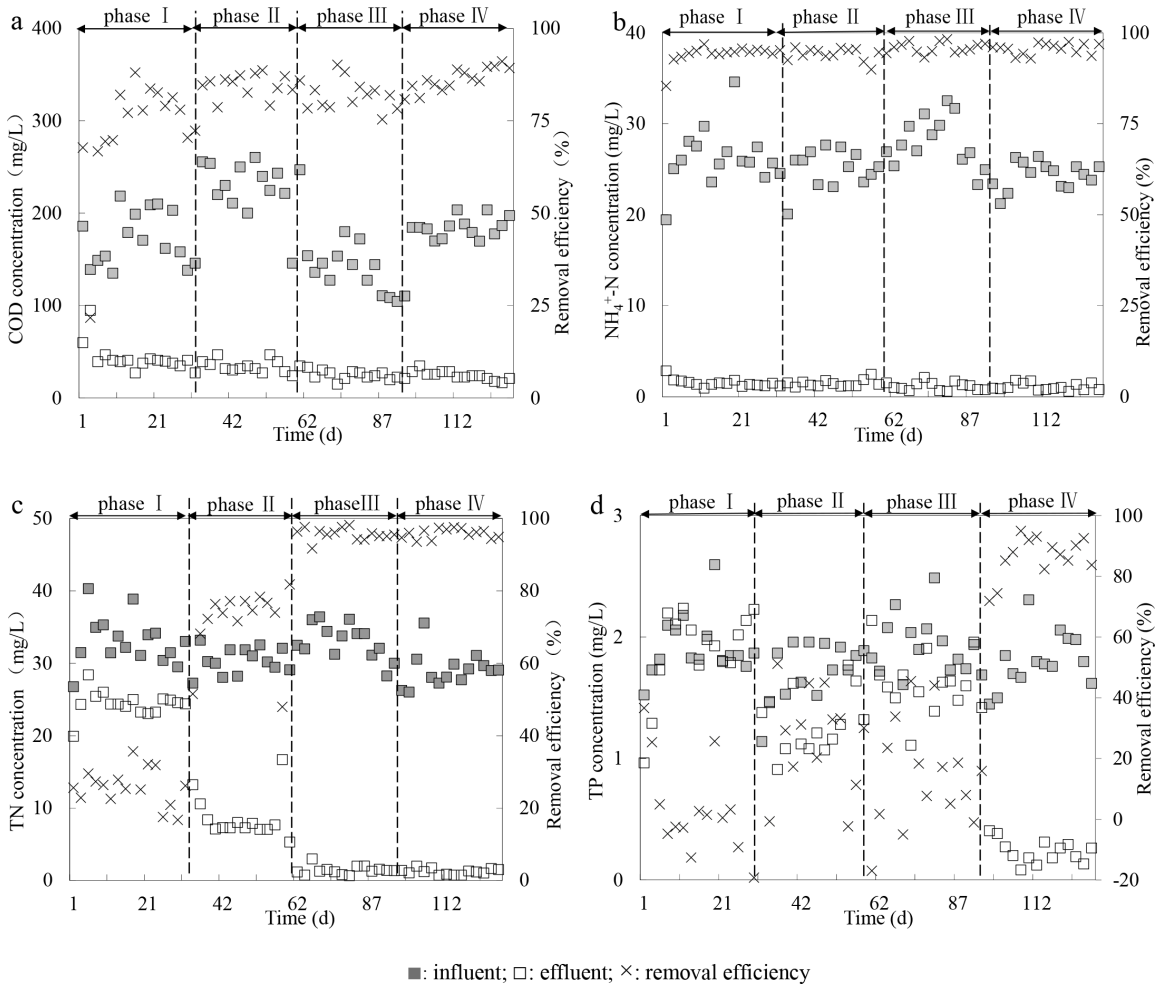

Fig. 2. Removal performance of COD, NH_4^+–N, TN and TP in the SDAO and EBPR-SAD systems during phases I–IV.

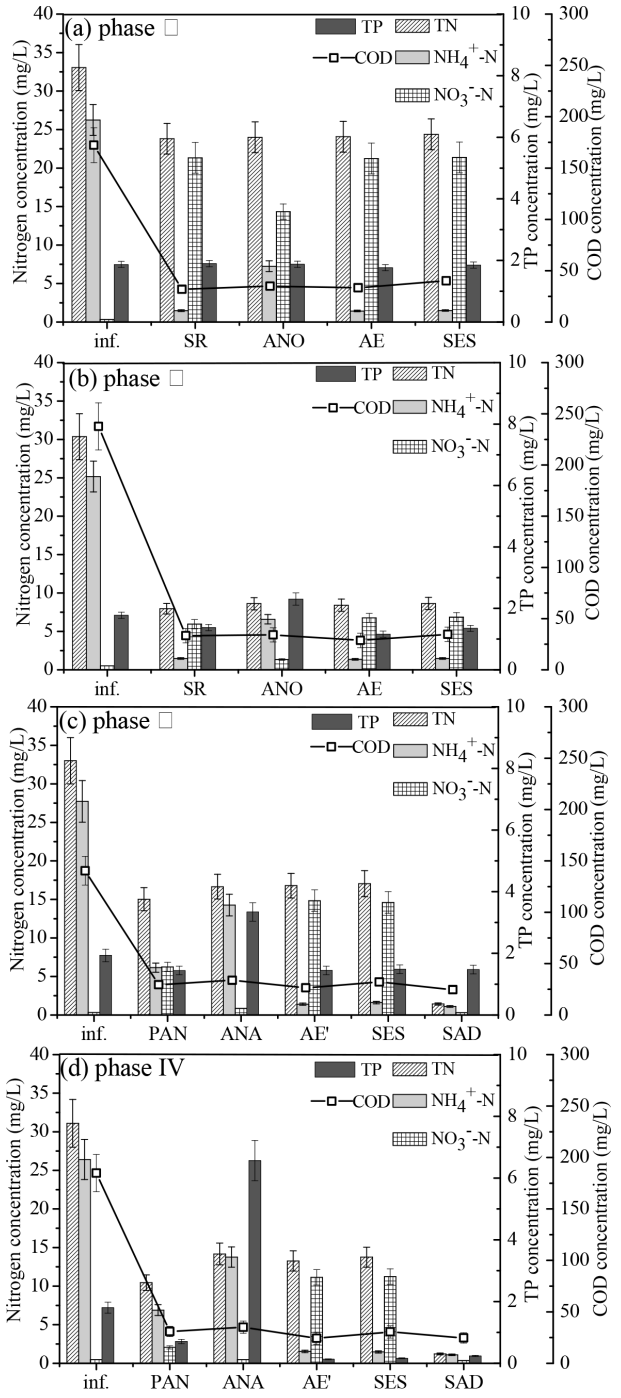

Fig. 3. Variations in COD, NH_4^+–N, TN, NO_3^-–N and TP in each zone of the SDAO system and the EBPR-SAD system during phases I–IV. (inf: influent; SR: sludge restore zone; ANO: anoxic zone; AE: aerobic zone in SDAO system; SES: secondary settler; PAN: pre-anoxic zone; ANA: anaerobic zone; AE': aerobic zone in EBPR-SAD system; SAD: sulfur autotrophic denitrification reactor.)

II. With added sodium acetate, the SDAO system effluent TN was reduced to 8.63 mg/L and the corresponding removal efficiency increased to 71.6%, as shown in Figs. 2(c) and 3(b). These results indicated that denitrification was weak in the anoxic zone because of limited organic carbon (C/N ratio 4–5). Adding sodium acetate was able to improve nitrogen removal performance appreciably in the SDAO system.

External organic carbon supplementation could improve nitrogen removal performance in the SDAO system; however, most organic carbon was used as a carbon source in the microbial assimilation process, which resulted in an increased cost of treating waste sludge. Furthermore, the nitrogen pollutant removal efficiency, which cannot reach 100%, was limited by the nitrate recycling ratio and sludge return ratio in the anoxic/aerobic process, even when the amount of influent organic carbon was sufficient. In addition, nitrate recycling consumed a large amount of kinetic energy for nitrate removal. Thus, the EBPR-SAD system was utilized to upgrade the SDAO system, the results of which, in terms of nitrogen removal, are shown in Figs. 2(c) and 3(c) and (d). The EBPR-SAD system's average effluent TN values were 1.42 and 1.21 mg/L, with corresponding removal efficiencies of 95.7% and 96.1% (Fig. 2(c)). The variations in TN and NO_3^-–N along the EBPR-SAD system during phases III–IV are shown in Figs. 3(c) and (d). The TN concentration throughout the EBPR process was approximately 14.7–17.0 mg/L, except in the PAN during phase IV, where it was 9.15 mg/L. The TN concentration in the PAN reached a lower level in phase IV because of the improvement in heterotrophic denitrification by the addition of sodium acetate (approximately 40 mg/L COD). Moreover, because of NO_3^-–N removal via heterotrophic denitrification, the NO_3^-–N concentrations in the PAN decreased to 6.23 mg/L (phase III) and 1.57 mg/L (phase IV) compared with those observed in the return sludge. Importantly, NO_3^-–N concentrations had noticeably decreased in phase IV before inflowing into the ANA, which was propitious for PAOs to release phosphorus. In the aerobic zone, NH_4^+–N was transformed into NO_3^-–N via nitrification processes, and the EBPR process effluent NO_3^-–N was approximately 12–15 mg/L, accounting for 86% of TN. Therefore, the SAD process was proposed to remove the NO_3^-–N that was not decomposed in the EBPR process. Finally, the NO_3^-–N concentration reached less than 1 mg/L in the SAD effluent. The results indicated that the EBPR-SAD system improved nitrogen removal performance, and those nitrogen pollutants were almost entirely removed during phases III–IV.

3.1.3. TP removal

The TP removal performances of the SDAO and EBPR-SAD systems were investigated during phases I–IV (Figs. 2(d) and 3(c and d)). In phases I–II, the influent TP was approximately 1.5–2.6 mg/L, with average corresponding SDAO effluent concentrations of 1.85 and 1.35 mg/L (Fig. 2(d)). The variations in TP throughout the SDAO system are presented in Figs. 3(a and b). The TP concentrations in the SDAO system, which were approximately 1.77–1.90 mg/L, were similar to those in the influent during phase I (Fig. 3(a)). With added sodium acetate (COD of 40 mg/L), phosphorus release was minimal in the anoxic zone, and the corresponding concentration increased to 2.31 on average; however, phosphorus removal was still inefficient (Fig. 3(b)). The results indicated that the performance of TP removal was subpar in the SDAO system, even when sodium acetate was added as an external

organic carbon source. Phosphorous release processes were not significant, and the concentration of TP did not change appreciably in the SDAO system.

During phases III–IV, the influent TP was 1.87 mg/L, on average, with corresponding EBPR-SAD system effluent concentrations of 1.48 mg/L (phase III) and 0.24 mg/L (phase IV). The variations in TP throughout the EBPR-SAD system are presented in Figs. 3(c) and (d). In phase III, TP concentration in the ANA increased to 3.34 mg/L, due to the release of phosphorus; however, TP removal was ineffective, with a removal efficiency of 23.6% (Fig. 3(a)). Although there was an ANA in the EBPR process, phosphorus release was weak during phase III. Therefore, sodium acetate (COD of 40 mg/L) was added into the influent to improve the performance of TP removal during phase IV. In phase IV, distinct phosphorus release was observed in the ANA, and the corresponding TP increased to 6.57 mg/L compared with the TP value of 3.34 mg/L in phase III. The removal efficiency of TP increased from 23.6% to 86.9%. The excellent TP removal is attributed to the following: first, the competition for organic carbon between denitrifying heterotrophs and PAOs was eliminated by efficient denitrification in the PAN; second, sodium acetate, which is a short-chain volatile fatty acid, was added into the influent and utilized by PAOs, allowing them to obtain enough organic carbon for the release of phosphorus in the ANA and enabling the TP concentration to decrease via PAOs' uptake process in the aerobic zone to an average of 0.13 mg/L. The results indicate that efficient phosphorous removal was achieved in the EBPR-SAD system with sodium acetate dosing.

3.2. The pollutant removal capacity and mechanisms in the SDAO and EBPR-SAD systems

Because of the effects of nitrate recycling, sludge return, and step feeds, the pollutant concentrations in the biotreatment units did not reveal the removal capacity. Therefore, based on materials balance, the pollutant removal capacity in the biotreatment units was calculated and is shown in Fig. 4.

3.2.1. COD removal capacity and mechanisms

The COD removal capacity in different zones of the SDAO and EBPR-SAD systems are presented in Fig. 4. In the SDAO system, COD was mainly consumed in the anoxic zone, as shown in Figs. 4(a) and (b); 76.1% and 80.7% COD were reduced in the anoxic zone. In contrast, COD was mainly consumed in the pre-anoxic and ANAs in the EBPR-SAD system. Because 30% influent flowed into the PAN, 25.7% (phase III) and 24.8% (phase IV) COD were utilized in that zone of the EBPR-SAD system; afterwards, 49.1% (phase III) and 50.8% (phase IV) COD were utilized in the ANA, as shown in Figs. 4(c) and (d). However, the COD removal capacity in the aerobic zone was low: 3.65%, 9.22%, 10.6% and 11.7% during phases I–IV. Notably, there was an average decrease of 7 mg/L COD in the SAD reactor, which may be attributed to the filtration by sulfur granules. The results indicate that COD was effectively consumed when flowing into the wastewater treatment system during phases I–IV. This finding could explain why the COD did not change much between the SDAO and EBPR-SAD system effluents, although influent

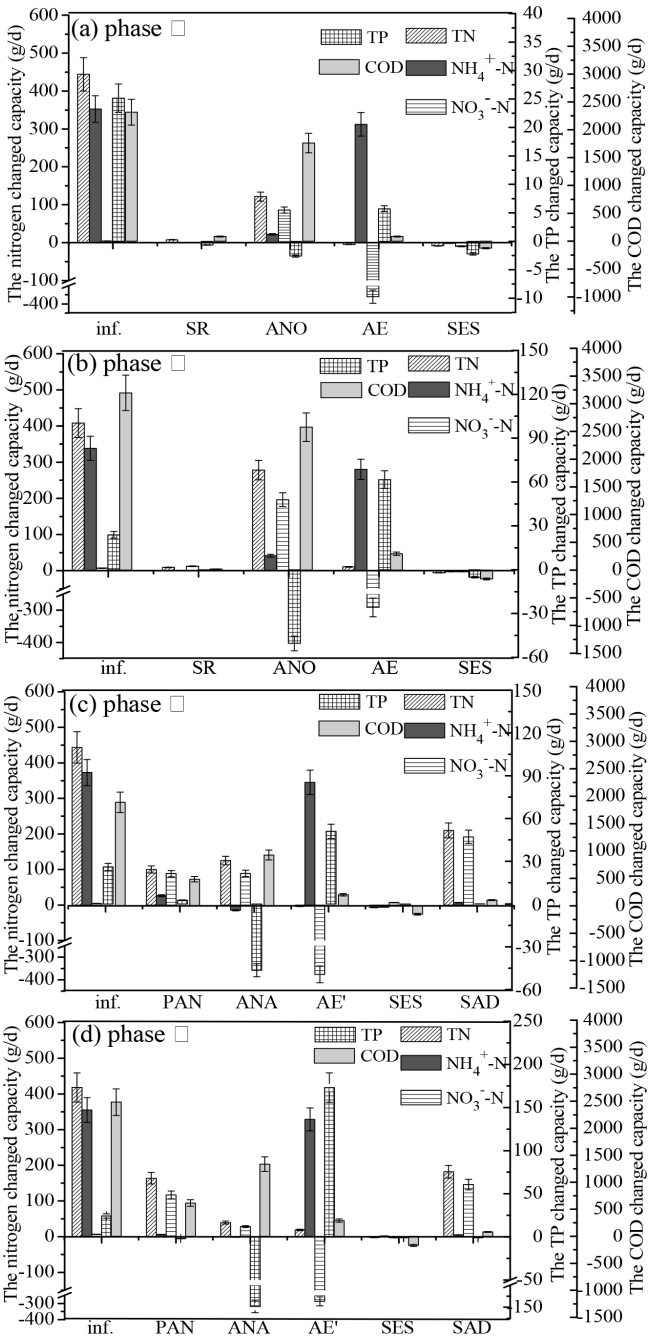

Fig. 4. Changed capacity of COD, NH_4^+–N, TN, NO_3^-–N and TP in each zone of the SDAO and EBPR-SAD systems during phases I–IV. (inf: represent total pollutant capacity in influent; SR: sludge restore zone; ANO: anoxic zone; AE: aerobic zone in SDAO system; SES: secondary settler; PAN: pre-anoxic zone; ANA: anaerobic zone; AE′: aerobic zone in EBPR-SAD system; SAD: sulfur autotrophic denitrification reactor; Note: positive and negative values stand for pollutants removal and release capacity, respectively.)

COD fluctuated between 104.6 and 260.3 mg/L. On the other hand, a small amount of COD was removed in the aerobic zone because the organic carbon was adequately oxidized before flowing into the aerobic zone, which would benefit the

metabolism of nitrifying bacteria and avoid inhibitory effects caused by excessive COD.

3.2.2. Nitrogen removal capacity and mechanisms

In the SDAO and EBPR-SAD systems, NH_4^+–N removal exhibited similar characteristics between phases I–IV. NH_4^+–N removal mainly occurred in the aerobic zone via nitrification, and the removal capacities were 312.1, 280.3, 345.4 and 328.5 g/d, with corresponding removal efficiencies of 88.5%, 82.8%, 92.7% and 92.6% during phases I–IV (Fig. 4). It was observed that wastewater treatment retained effective NH_4^+–N removal after modification from the SDAO to the EBPR-SAD system. The reason may be that the COD was effectively consumed before flowing into the aerobic zone, providing an advantage to nitrifying bacteria metabolism and avoiding inhibitory effects caused by excessive COD concentrations.

In the SDAO system, TN removal and denitrification mainly occurred in the anoxic zone. The removal capacities of TN in the anoxic zone were 126.8 and 278.0 g/d with removal efficiencies of 31.6% and 68.1% during phase I–II, as shown in Figs. 4(a) and (b). The results indicate that nitrogen removal performance was ineffective in the anoxic zone of the SDAO system because of a shortage of organic carbon during phase I. Adding sodium acetate clearly improved nitrogen removal performance in the anoxic zone, with the NO_3^-–N concentration decreasing from 14.3 to 1.34 mg/L.

In the EBPR-SAD system, TN removal via denitrification mainly occurred in the PAN, ANA and SAD reactor in phase III, and in the PAN and SAD reactor in phase IV, as shown in Figs. 4(c) and (d). The TN removal capacities in the PAN, ANA and SAD reactor were 99.9 g/d (22.5%), 125.2 g/d (28.2%) and 210.0 g/d (47.3%), respectively, in phase III, and 163.8 g/d (39.2.4%), 39.5 g/d (9.43%) and 182.0 g/d (43.5%), respectively, in phase IV. In phase III, nitrate carried by return sludge was not effectively removed in the PAN because of a shortage of electron donors; afterwards, the residual nitrate was removed in the ANA. With the dosing of sodium acetate in phase IV, denitrification was enhanced in the PAN, and the NO_3^-–N concentration decreased from 6.23 (phase III) to 1.57 mg/L (phase IV). The TN concentration of the EBPR process effluent was reduced from 17.0 in phase III to 14.8 mg/L in phase IV. On the other hand, nitrate constituted a major proportion of the effluent TN in the EBPR process. Therefore, the SAD process was proposed to further enhance nitrate removal. The SAD effluent concentration of TN decreased to 1.42 mg/L (phase III) and 1.21 mg/L (phase IV). These results indicate that the EBPR-SAD system was capable of achieving efficient nitrogen removal as long as the NH_4^+–N was sufficiently oxidized. In the EBPR-SAD system, heterotrophic denitrification occurred in the EBPR process and used organic carbon as an electron donor to remove nitrate. Afterwards, residual nitrate was removed via the SAD process, which used sulfur as an electron donor.

3.2.3. Phosphorous removal capacity and mechanisms

In the SDAO system, phosphorous removal performance was inefficient. Fig. 4(a) shows that the phosphorus release process was not observed and that the concentration of TP did not change appreciably in phase I. With sodium acetate dosing in phase II, the capacity of phosphorus release was increased to 50.5 g/d in the anoxic zone, which resulted in only 23.9% TP removal in the SDAO system, as shown in Fig. 4(b). The reasons for the inefficient phosphorous removal in the SDAO system are summarized as follows: first, there was an absence of an ANA, and nitrate existed in the anoxic zone, which resulted in competition for organic carbon between denitrifying heterotrophs and PAOs; second, organic carbon levels were insufficient for the phosphorus release process. When sodium acetate was added into the influent, the denitrification performance improved, and NO_3^-–N was decreased to 1.34 mg/L in the anoxic zone. Residual organic carbon was utilized by PAOs, and a slight amount of phosphorous was released in the anoxic zone, which resulted in 23.9% TP removal. This finding indicated that the organic carbon level was insufficient for phosphorus release processes. With enough organic carbon, denitrification and phosphorous release processes would occur consecutively in a single reactor.

In phase III, phosphorus release was weak, although there was an ANA in the EBPR process; the performance of phosphorus removal was disappointing. The capacity of anaerobic phosphorus release was 46.4 g/d, accounting for 178.8% of influent TP. Afterwards, the aerobic zone phosphorus uptake was 51.0 g/d, and the EBPR-SAD system effluent TP concentration was 1.48 mg/L with a corresponding removal efficiency of 23.6%, as shown in Fig. 4(c). Because of insufficient organic carbon, residual NO_3^-–N (6.23 mg/L) in the PAN flowed into the ANA, which resulted in competition for the organic carbon between denitrifying heterotrophs and PAOs. Therefore, the PAOs could not obtain enough organic carbon for phosphorus release, which led to unsatisfactory phosphorus removal in phase III. During phase IV, sodium acetate (COD of 40 mg/L) was added into the influent to improve phosphorus removal. The PAN effluent concentration of NO_3^-–N was reduced to 1.57 mg/L, which resulted in the elimination of competition for organic carbon between denitrifying heterotrophs and PAOs. Therefore, PAOs could obtain enough organic carbon for phosphorus release in the ANA. Distinct phosphorus release was observed in the ANA, and the capacity of anaerobic phosphorus release increased to 148.1 g/d, accounting for 610.7% of the influent TP, as shown in Fig. 4(d). Afterwards, 173.0 g/d phosphorus was removed in the aerobic zone, and the effluent concentration of TP in the EBPR-SAD system decreased to 0.24 mg/L, with a corresponding removal efficiency of 86.9%. The results indicate that the absence of nitrate and uptake of organic carbon were important for phosphorus release in the ANA. Moreover, phosphorus release played an important role in the phosphorus uptake process.

3.3. Bacterial community in the SDAO and EBPR-SAD systems

3.3.1. Richness and diversity of bacterial communities

The number of OTUs, Good's coverage, ACE, Chao1 and the Shannon diversity index (at a cut-off level of 3%) were calculated for the SDAO and EBPR-SAD systems and are summarized in Table 1. The results show that the Good's coverage ranged from 88.7% to 90.5% in the SDAO

Table 1
Richness and diversity estimators of the microbial community in the SDAO and EBPR-SAD system

Samples	OTU	Good's coverage	Chao1	ACE	Shannon
SR	3,914	0.905	9,367	14,887	6.44
ANO	3,622	0.900	9,496	15,401	6.13
AE	3,710	0.887	9,205	15,150	6.37
PAN	5,540	0.858	14,731	24,261	6.98
ANA	4,771	0.876	10,965	17,803	6.86
AE'	5,174	0.886	12,260	19,610	6.84
SAD	5,275	0.857	13,433	22,012	7.07

system and from 85.7% to 88.6% in the EBPR-SAD system, indicating that most bacterial communities in the two wastewater treatment systems were detected in the libraries. The numbers of OTUs were 3,914 (sludge recovery zone), 3,622 (anoxic zone), and 3,710 (aerobic zone) in the SDAO system and 5,540 (PAN), 5,771 (ANA), 5,174 (aerobic zone), and 5,275 (SAD reactor) in the EBPR-SAD system. The patterns of the Chao1 and ACE values in the EBPR-SAD system were higher than those found in the SDAO system, as shown in Table 1. Based on the OTUs, Chao1 and ACE, the results demonstrate that the EBPR-SAD system has richer diversity than the SDAO system. Previous studies have shown that the type of wastewater biotreatment system, influent substrate, temperature, geographical position, etc., may affect the diversity of bacterial communities [22,27,28]. Diversity further affects the performance of wastewater biotreatment systems. The type of wastewater biotreatment system in the SDAO system was altered when upgrading to the EBPR-SAD system; in particular, an ANA was established in the EBPR process, which provided conditions for creating richer diversity. The performance of the EBPR-SAD system was better than that of the SDAO system, possibly because of the diversity of bacterial colony establishment and the more diverse biochemical reactions that were carried out by these bacterial communities.

Based on the UniFrac metric, CA and PCoA were conducted to evaluate the similarities of bacterial communities between the SDAO and EBPR-SAD systems. As shown in Fig. 5(a), CA revealed that the bacterial communities in seven sludge samples from the SDAO and EBPR-SAD systems could be clustered into three groups: (1) Group I contained the three sludge samples from the SDAO system; (2) Group II was the sludge sample from the SAD reactor; (3) Group III contained the three sludge samples from the EBPR process. In addition, the PCoA results, which were consistent with the CA results, showed that the three sludge samples from the SDAO system tended to cluster together and that the three sludge samples from the EBPR process tended to cluster together; however, the sludge sample from the SAD reactor was clearly different from the samples from the SDAO and EBPR processes (Fig. 5(b)). Based on the CA and PCoA results, it was clear that there would be significant differences between the SDAO, EBPR and SAD processes, although the characteristics of the wastewater and operational parameters were similar between those systems. The results demonstrate that bacterial communities were affected by the type of wastewater biotreatment system.

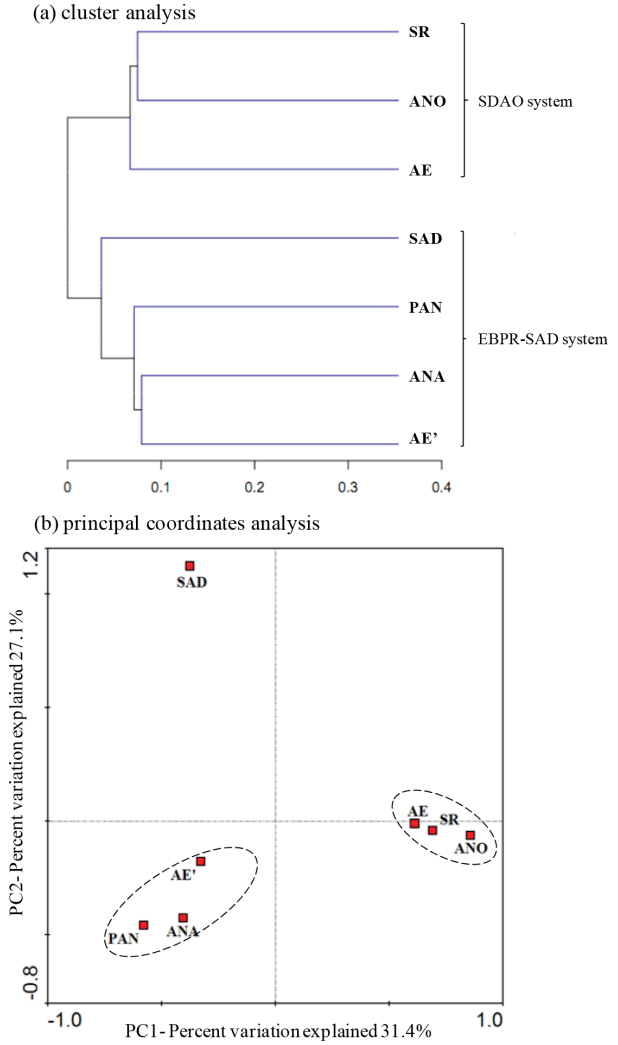

Fig. 5. Beta diversity for the SDAO and EBPR-SAD systems. (a) cluster analysis; (b) 2-D principal coordinates analysis. SR, ANO and AE: sludge recovery zone, anoxic zone and aerobic zone in the SDAO system; PAN, ANA, AE' and SAD: pre-anoxic zone, anaerobic zone, aerobic zone and sulfur autotrophic denitrification reactor in the EBPR-SAD system.

3.3.2. Bacterial taxonomic identification

The effective bacterial sequences in seven sludge samples from the SDAO and EBPR-SAD systems were assigned to

taxonomies by the RDP Classifier at an 80% threshold. At the phylum level, 25 bacterial phyla were detected in the SDAO and EBPR-SAD systems. Nine of those phyla were the predominant ones (>1% abundance in any sludge samples), accounting for 87.0%–90.7% of the effective sequences, as shown in Fig. 6(a). *Proteobacteria* was the most abundant phylum in the SDAO and EBPR-SAD systems, accounting for 42.2%–59.1% of the total effective sequences. The results indicate that *Proteobacteria* displayed considerably higher abundance in both the SDAO and EBPR-SAD systems, consistent with results obtained for bacterial communities in active sludge [28]. The other dominant phyla were *Bacteroidetes* (7.07%–16.9%), *Planctomycetes* (6.65%–16.1%), *Chloroflexi* (1.69%–12.4%) and *Firmicutes* (1.80%–15.3%), as observed in previous studies [22,24]. One study reported that *Chloroflexi* was important in anaerobic digestion sludge [29], which may explain why the abundance of *Chloroflexi* in the EBPR-SAD system was not only higher than that in the SDAO system but also higher in the ANA and SAD reactor in the EBPR-SAD system. However, the abundance of *Planctomycetes* in the EBPR-SAD system was higher than that in the SDAO system and higher than that observed in previous studies [22,28]. These five phyla (>1% abundance in all sludge samples) accounted for 81.7%–87.0% of the total effective sequences in the SDAO and EBPR-SAD systems, followed by four other major phyla (>1% abundance in least one sludge sample): *Verrucomicrobia* (0.33%–3.19%), *Armatimonadetes* (0.15%–1.64%), *Gemmatimonadetes* (0.23%–1.42%) and *Nitrospirae* (0.12%–1.09%).

At the class level, 53 bacterial classes were detected in the SDAO and EBPR-SAD systems, with 18 of them being dominant classes (>1% in at least one sample), as shown in Fig. 6(b). These 18 classes accounted for 86.8%–90.3% of the total effective sequences. Among the 18 classes, *Sphingobacteriia*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Planctomycetacia* were commonly shared by all sludge samples from both the SDAO and EBPR-SAD systems, accounting for 60.4%–73.3% of the total effective sequences. *Armatimonadia*, *Bacilli* and *Verrucomicrobiae*, each observed in at least 1% abundance, were found in the SDAO system, and *Deltaproteobacteria*, *Caldilineae*, *Anaerolineae* and *Phycisphaerae*, with at least 1% abundance, appeared in the EBPR-SAD system. A few classes, including *Nitrospira*, *Gemmatimonadetes*, *Flavobacteriia*, *Actinobacteria*, *Cytophagia* and *Clostridia*, with more than 1% abundance appeared in one or more units in the SDAO or EBPR-SAD system. Within *Proteobacteria*, *Alphaproteobacteria* (5.06%–35.9%, average 19.4%) and *Betaproteobacteria* (10.4%–28.8%, average 16.3%) were the most dominant classes in the two wastewater biotreatment systems, followed by *Gammaproteobacteria* (5.72%–14.9%, average 10.2%) and *Deltaproteobacteria* (0.85%–6.17%, average 2.30%). The results indicated that *Alphaproteobacteria* was the most abundant in the two treatment systems, followed by *Betaproteobacteria*. This finding is unlike the results obtained for bacterial communities using 454-pyrosequencing [22], which showed that *Betaproteobacteria* was the most dominant *Proteobacteria*. However, the findings in this study are similar to other results [24] indicating that *Alphaproteobacteria* was the most predominant *Proteobacteria*. In addition, *Sphingobacteriia* and *Planctomycetacia* were subdominant classes, constituting 6.8%–14.9% (average 11.0%) and 6.12%–12.2% (average 9.37%) of the total effective sequences.

At the order level, 108 bacterial orders were detected in the SDAO and EBPR-SAD systems, 26 of which were predominant orders (>1% in at least one sample), accounting for 82.0%–86.7% of the total effective sequences, as shown in Fig. 6(c). The top five orders were *Sphingobacteriales* (6.80%–14.9%, average 10.9%), *Rhizobiales* (2.38%–16.0%, average 9.68%), *Planctomycetales* (6.12%–12.2%, average 9.37%), *Burkholderiales* (6.60%–11.8%, average 9.07%) and *Rhodobacterales* (0.86%–17.7%, average 7.29%). At the family level, 211 bacterial families were identified in the SDAO and EBPR-SAD systems, 34 of which were dominant (>1% in at least one sample), as shown in Fig. 6(d). These 34 abundant families accounted for 79.4%–86.1% of the total effective sequences. The top five families were *Planctomycetaceae* (6.12%–12.2%, average 9.37%), *Comamonadaceae* (6.16%–11.4%, average 8.62%), *Rhodobacteraceae* (0.86%–17.7%, average 7.29%), *Moraxellaceae* (2.08%–13.4%, average 6.88%) and *Saprospiraceae* (2.37%–10.2%, average 5.91%).

A total of 592 genera were identified in the SDAO and EBPR-SAD systems, with 84 commonly shared by all seven sludge samples from the SDAO and EBPR-SAD systems and accounting for 37.2%–58.8% of the total effective bacterial sequences. Moreover, among these 595 genera, 28 genera were dominant (>1% in at least one sample), accounting for 35.6%–65.4% of the total effective sequences, which were selected and compared with their abundances in other samples, as shown in Fig. 6(c). Among the 28 genera, *Pirellula*, *Acidovorax*, *Planctomyces* and *Azospira* were dominant in both the SDAO and EBPR-SAD systems; *Methylocystis* and *Hyphomicrobium* were dominant in the SDAO system and the EBPR process; *Rhodovulum*, *Carnobacterium*, *Segetibacter*, *Defluviimonas*, *Psychrobacter*, *Mesorhizobium*, *Roseibacillus* and *Armatimonas* were abundant in the SDAO system; *Acinetobacter*, *Gemmata*, *CL500-3* and *SM1A02* were abundant in the EBPR process; *Lysobacter*, *Ferritrophicum*, *Thiobacillus*, *Georgfuchsia* and *Desulfovibrio* were only abundant in the SAD reactor; and *Atopococcus*, *Smithella*, *Solitalea*, *Pseudomonas* and *Nitrospira* were abundant in at least one sample from the SDAO system or EBPR process.

3.3.3. Relationships between pollutant removal and community structure

RDA analysis was performed to reveal the relationship between the abundance of bacterial phyla and environmental parameters in the SDAO and EBPR-SAD systems (Fig. 7). The results of RDA analysis were explained according to previous studies [30,31].

Fig. 7(a) shows that the first and second canonical axes had 74.1% and 25.9% variation, respectively, indicating that significant interrelations were observed among the bacterial community composition and the pollutant removal/environmental conditions in each treatment zone [32]. RDA analysis showed that the phylum of *Firmicutes* was related to the NH_4^+–N, TP concentration, COD and TN removal in the anoxic zone of the SDAO system. The phyla of *Bacteroidetes* and *Gemmatimonadetes* were related to the NO_3^-–N concentration, and *Proteobacteria* was correlated with NH_4^+–N removal in the aerobic zone. Because sewage water flows into the anoxic zone with phosphorus release, NH_4^+–N and

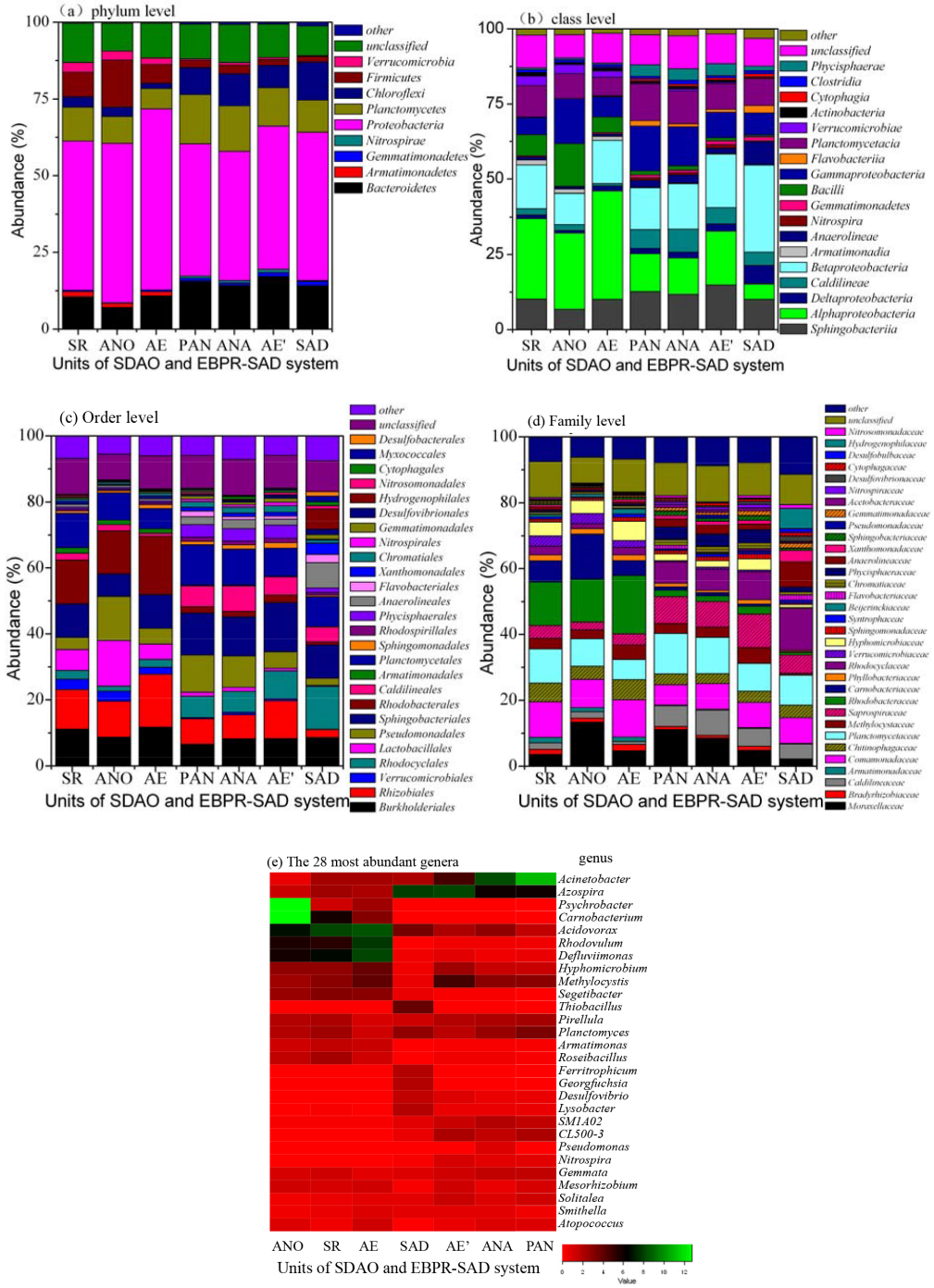

Fig. 6. Distributions of bacteria in the SDAO and EBPR-SAD systems at the (a) phylum level, and (b) class level, (c) order level, (d) family level and (e) a heat map of the 28 most abundant genera. Taxa represented had an occurrence of >1% frequency in at least one sample. SR, ANO and AE: sludge recovery zone, anoxic zone and aerobic zone in the SDAO system; PAN, ANA, AE' and SAD: pre-anoxic zone, anaerobic zone, aerobic zone and sulfur autotrophic denitrification reactor in the EBPR-SAD system.

TP concentration positively correspond to the anoxic zone, whereas COD and TN removal were positively correlated with the anoxic zone because of pollutant biodegradation and denitrification. NO_3^-–N removal was more passively affected by the aerobic zone than positively related to the anoxic because most of the NH_4^+–N was converted to NO_3^-–N by the nitrification process in the aerobic zone. Nevertheless, denitrification was inefficient in the anoxic zone because of electron donor shortage. On the other hand, the NO_3^-–N concentration had a mixed correlation with sludge recovery and the aerobic zone because the NO_3^-–N in the return sludge was decomposed when it flowed into the sludge recovery zone.

Fig. 7(b) shows that the first and second canonical axes accounted for 61.7% and 31.6% of the variation in the

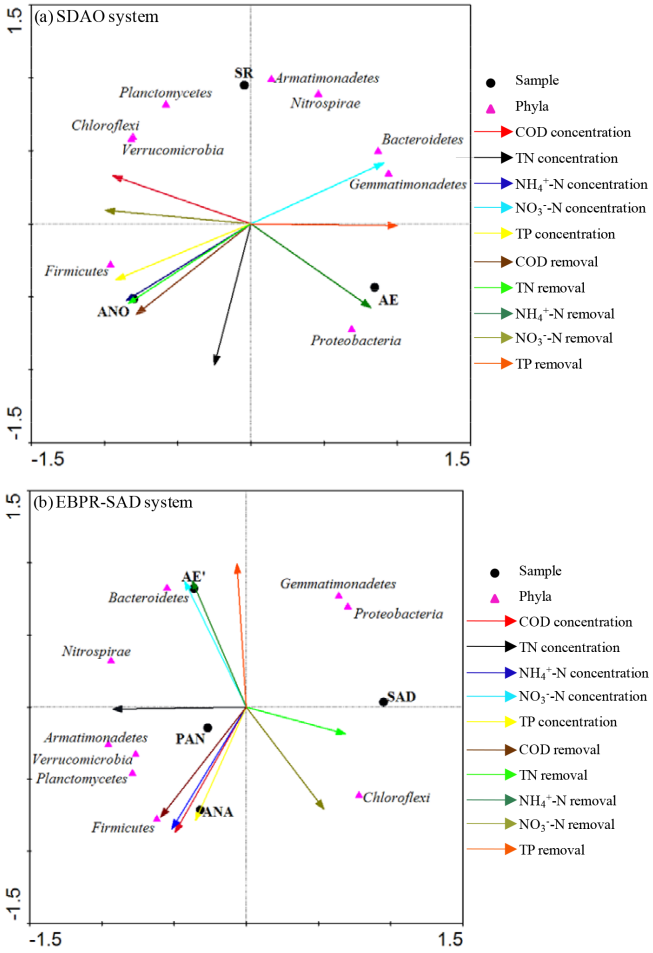

Fig. 7. Redundancy analysis of the relationship between the abundance of phylum and environmental parameters. (a) SDAO system; (b) EBPR-SAD system. SR, ANO and AE: sludge recovery zone, anoxic zone and aerobic zone in SDAO system; PAN, ANA, AE′ and SAD: pre-anoxic zone, anaerobic zone, aerobic zone and sulfur autotrophic denitrification reactor in EBPR-SAD system.

EBPR-SAD system. Triplet analysis showed that the phylum *Firmicutes* was related to the environmental parameters that existed in the pre-anoxic and ANAs, such as COD, TN, TP, NH_4^+–N concentration and COD removal. The result was similar to that obtained for the SDAO system, indicating that *Firmicutes* was related to the metabolic processes carried out in the anaerobic environment. Li et al. [33] and Liu et al. [34] also showed that *Firmicutes* was a significant phylum in anaerobic environments; only the relationship was different in degree. *Bacteroidetes* was positively correlated with NH_4^+–N removal, NO_3^-–N concentration and TP removal in the aerobic zone, while *Bacteroidetes* was interrelated to O_1 and A_2 in two-stage A/O-MBR system [34]. *Chloroflexi* was related to NO_3^-–N removal. Similarly to the anoxic zone in the SDAO system, phosphorus release and COD removal were positively correlated with the ANA in the EBPR-SAD system. However, TN removal showed mixed correlations with the pre-anoxic, ANA and SAD process because denitrification can be achieved in those zones, unlike in the SDAO system. Moreover, a previous study showed that TN removal was positively related to the pre-anoxic, anoxic and SAD processes in a JHB-SAD system [33]. The different results indicate that the transformation of wastewater treatment system led to a change in the nitrogen removal zone. NH_4^+–N removal, NO_3^-–N concentration and TP removal were positively related to the aerobic zone because of nitrification and phosphorus uptake in the EBPR-SAD system, consistent with the results of a previous study [33]. In addition, TP removal was more positively related to the aerobic zone than in the SDAO system because phosphorus uptake was better in the EBPR-SAD system. In the EBPR-SAD system, NO_3^-–N in the return sludge was efficiently removed in the PAN such that afterwards, phosphorus release was excellent compared with that in the SDAO system, resulting in more efficient TP removal in the EBPR-SAD system than in the SDAO system.

4. Discussion

Based on the nutrients removal performance of the SDAO and EBPR-SAD systems, the following conclusions are drawn. Comparison of phases I and II indicated that an electron donor is necessary for nitrate removal via denitrification. Comparison of phases III and IV demonstrated that the absence of nitrate from the ANA and sufficient carbon sources for phosphorus removal are requisite for phosphorus release. Comparison of phases I and III proved that the prolonged HRT of the anaerobic and anoxic zones is beneficial for nitrate removal via denitrification. Comparison of phases II and IV showed that the ANA is required for phosphorus release.

Table 2 compares the EBPR-SAD system and other similar systems in terms of nutrients removal performance. The EBPR-SAD system's performance is attractive when treating low C/N ratio municipal wastewater. Moreover, nitrate recycling and sludge return can be combined as long as the nitrogen efficiency meets discharge requirements. The reasons are as follows: (1) the sludge return ratio must increase to maintain the sludge content of the wastewater treatment system when treating low-pollution wastewater; (2) the main obstacle to nitrogen removal is a shortage of electron donors when treating low C/N ratio municipal wastewater, and a high recycling ratio is not required. Importantly, power consumption is reduced when combining nitrate recycling and sludge return. Furthermore, it is recommended that heterotrophic denitrification be carried out in front of the ANA. The nitrate in sludge return must be efficiently removed; the mixture can then flow into the ANA to ensure a strict anaerobic environment for phosphorus release.

When the SAD process is applied in a wastewater biotreatment system, there is an alternative pathway for removing nitrogen from low C/N ratio municipal wastewater. Therefore, organic carbon in the influent can be preferentially distributed to PAOs for phosphorus release. Moreover, an integrated anaerobic/anoxic/aerobic SAD process, such as that in the EBPR-SAD system, achieves near-complete nitrogen removal. On the other hand, WWTPs generally feature a filtering process for SS removal. If nitrogen removal is inefficient because of a shortage of organic carbon, WWTPs only require a change in filter media to sulfur particles; efficient

Table 2
Comparison of the documented efficiency of other low C/N ratio nutrient removal processes

Wastewater source	Influent COD (mg/L)	Influent TN (mg/L)	Influ-ent TP (mg/L)	COD/TN ratio	Wastewater treatment process	Effluent COD (mg/L)	Effluent TN (mg/L)	Effluent TP (mg/L)	Removal efficiency of TN (%)	Removal efficiency of TP (%)	References
Municipal wastewater	156	40.06	3.59	3.78	Modified A^2O-MBR	Below 30	18.51	2.67	52.1	25.5	[35]
Municipal wastewater	156 with NaAC	40.06	3.59	5.12	Modified A^2O-MBR	Below 30	Below 15	2.02	74.9	43.5	[35]
Actual wastewater	201.6 ± 19.7	39.7 ± 4.3	39.7 ± 4.3	Below 5.2	Modified A^2O	26.7 ± 16.8	13.9 ± 1.27	0.28 ± 0.05	65	93	[36]
Real and Synthetic wastewater	543	92	10.1	5	UCT-IFAS-MBR	Below 20	–	3.35	53	67	[37]
Municipal wastewater	387.2	73.3	5.8	5.28	A^2O-BAF	39.3	9.5	0.1	87.0	98.3	[19]
Municipal wastewater	129.4	29.1	1.70	4.45 on average	JHB-SAD	20.5	1.42	1.77	95.1	–	[33]
Municipal wastewater	112.7 with NaAc (COD of 40 mg/L)	31.6	1.72	5.0–6.5	JHB-SAD	22.7–29.5	1.4	0.17	95.2	90.1	[33]
Municipal wastewater	140.4	32.5	1.87	4.32 on average	EBPR-SAD	24.6	1.42	1.48	95.7	23.6	This study phase III
Municipal wastewater	134.8 with NaAc (COD of 40 mg/L)	30.2	1.87	5.0–6.5	EBPR-SAD	24.6	1.21	0.24	96.1	86.9	This study phase IV

nitrogen removal can then be achieved. Therefore, SAD can be easily implemented in WWTPs for treating low C/N ratio municipal wastewater.

5. Conclusions

The main conclusions of this study are summarized as follows: (1) The EBPR-SAD system could achieve maximum removal efficiencies for COD, TN, NH_4^+–N and TP of 86.7%, 96.1%, 95.9% and 86.9%, respectively, with final concentrations in the system effluent of 24.6, 1.21, 1.10 and 0.24 mg/L, respectively. (2) Near-complete nitrogen removal could be achieved by the EBPR-SAD system when NH_4^+–N was sufficiently oxidized, an attractive feature for removing nitrogen from municipal wastewater with a low C/N ratio. (3) Based on the CA and PCoA results, it was clear that there would be significant differences between the SDAO, EBPR and SAD processes, indicating that the bacterial communities would be affected by the type of wastewater biotreatment system. (4) Taxonomic identification revealed that the phyla *Proteobacteria*, *Bacteroidetes*, *Planctomycetes*, *Chloroflexi*, *Firmicutes*, *Verrucomicrobia*, *Armatimonadetes*, *Gemmatimonadetes* and *Nitrospirae* constituted the majority of bacteria in the EBPR-SAD system. (5) RDA analysis showed that the incidence of the phylum *Firmicutes* was related to the environmental parameters in the pre-anoxic and ANAs. *Bacteroidetes* was positively correlated with NH_4^+–N removal, NO_3^-–N concentration and TP removal in the aerobic zone, and *Chloroflexi* was related to NO_3^-–N removal.

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Symbols

EBPR-SAD	—	Enhanced biological phosphorus removal-sulfur autotrophic denitrification
SDAO	—	Shenyang Degremont Anoxic Oxic process
SR	—	Sludge restore zone
ANO	—	Anoxic zone
AE	—	Aerobic zone
SES	—	Secondary settler
ANA	—	Anaerobic zone
PAN	—	Pre-anoxic zone
AHRT	—	Actual hydraulic retention time

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